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Detection and Typing of Human Papillomavirus in Biopsy and Cytological Specimens by Polymerase Chain Reaction and Restriction Enzyme Analysis: A Method Suitable for Semiautomation

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Screening for high-risk human papillomavirus (HPV) types allows the detection of women at a high risk of cervical squamous carcinomas, thereby defining a subset of patients targeted for more intensive screening and follow-up. Thirty-four cervical biopsy specimens and isolated cells from cervical smears of normal women or women diagnosed with high-grade intraepithelial lesion (HGSIL) were screened for the presence of HPV by in situ hybridization (ISH) and/or by polymerase chain reaction (PCR). The exact HPV type was determined using a novel restriction typing method. The detection of HPV was facilitated greatly by the use of a PCR-enzyme-linked immunosorbent assay (ELISA)-based method. HPV was detected by PCR in 32% of the biopsy specimens, whereas only 23% had a positive staining by ISH. In one case, a double infection was detected by ISH as well as by PCR. In two cases, the presence of HPV was detected by both methods but the exact type was different. Analyzing cells isolated from cervical smears by the PCR-ELISA technique or by PCR followed by agarose gel electrophoresis, HPV was detected only in patients with HGSIL and not in the control group. The PCR system is more sensitive than conventional ISH, and the PCR-ELISA system presented in this study is efficient in screening large series of cytological samples. Furthermore, this system allows exact HPV typing on the microtiter plate. These innovations may allow the application of HPV detection and typing as a routine screening method to identify patients with a high risk of developing cervical neoplasia.

KEY WORDS: human papillomavirus, PCR, PCR-ELISA, in situ hybridization, microtiter plate assay, isolation of cells from cervical smear, HPV typing, automation of HPV detection

INTRODUCTION

Growing evidence accumulated in the last years suggests that human papillomaviruses (HPV) play an important role in the pathogenesis of cervical, vaginal, and vulvar squamous carcinoma [Galloway and McDougall, 1989; zur Hausen, 1989; Pfister, 1987; Gissman, 1984; Reid et al., 1982]. HPV DNA is found in about 90% of cervical carcinomas and in 50% of vulvar malignancies [Galloway and McDougall, 1989; Pfister, 1987]. Currently, more than 70 different HPV types are known [DeVilliers, 1994]. The genital HPV types are grouped into "low-risk" (HPV-6,11,40,42,43,44,57) and into "high-risk" (HPV-16,18,30,31,33,35,39,45,51,52, 56,58,59,66) categories [Schneider, 1994]. Sequence comparison, phylogenetic tree analysis, and the different oncogenic potential of each HPV type support this classification [Delius and Hofmann, 1994; Lechner and Laimins, 1994]. Molecular genetic analysis showed that two HPV-encoded proteins, namely E6 and E7, are invariably expressed in carcinomas and tumor-derived cell lines [Schneider-Gadicke and Schwarz, 1986; Smotkin and Wettstein, 1986]. The viral oncoproteins E6 and E7 in particular interact with products of tumor suppressor genes, namely E6 of the high-risk HPVs with p53 [Crook and Vousden, 1994; Werness et al., 1990; Lechner and Laimins, 1994] and E7 with the retinoblastoma protein [Dyson et al., 1989], suggesting that this interaction might be related to the oncogenic properties of the virus.

A study by Koutsky et al. [1992] demonstrated that the risk was much higher for developing a high-grade SIL lesion, if an HPV-16 or 18 infection was detected compared to women without an HPV infection. This relation and the observation that HPV-infected tissue does not always show cytological abnormalities [Lambropoulos et al., 1994; Veress et al., 1994; DeVilliers et al., 1987] make it necessary to detect the HPV by mo-

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lecular genetic methods and to determine the exact HPV type. To date several different methods have been published for detection and typing of HPVs [reviewed by Roman and Fife, 1989].

The traditional and earliest method used to detect specific HPV types in invasive squamous cell carcinomas and several cervical cancer precursors was Southern blot hybridization [Dürst et al., 1985; Gissman et al., 1982, 1983; Gissman and zur Hausen, 1980]. This technique has the advantage of providing information about the conformation of the virus within the cell, i.e., whether the virus is integrated into the host DNA or episomal. The clear disadvantage of this method is that the procedure is time consuming and that large quantities of high-quality DNA (10 μg) are needed. Another widely used method is in situ hybridization (ISH) using *type-specific* probes to detect the virus. The major advantage of this method is the cellular localization of the virus. Studies using HeLa cells, which carry about 20 copies of HPV-18 in the genome, showed that the ISH requires a higher viral load (≥50 copies per cell) [Bernard et al., 1994a; Popper et al., 1994], suggesting that this method is not very sensitive. A method which circumvents the problem of sensitivity but still provides information about the cellular localization of the virus is in situ polymerase chain reaction (PCR) [Bernard et al., 1994a; Nuovo et al., 1991]. However, as a routine screening procedure this method may not be applicable.

PCR itself, a very sensitive method to detect foreign DNA, can be used to examine a larger series of samples in a reasonable time. This method has been used widely to detect HPV in fresh, frozen, or paraffin-embedded tissue [Adams et al., 1995; Monk et al., 1994; Popper et al., 1994; Lucotte et al., 1993; Bauer et al., 1991]. In this study, PCR was used to detect HPV and a novel method in determining the exact HPV type is described. Furthermore, this method is applicable to fresh and formalin-fixed paraffin-embedded tissue material and this assay is transferable to a microtiter plate-based detection and typing system.

MATERIALS AND METHODS

DNA Isolation From Formalin-Fixed Paraffin-Embedded Tissue

DNA was extracted from formalin-fixed, paraffin-embedded tissues by Proteinase K digestion. Successful amplification of a β-globin fragment indicated that the samples were adequate for PCR analysis and that no inhibitors were present [Bauer et al., 1991].

Isolation of Cells From Cervical Smears

Cervical smears of 22 patients, 11 normal and 11 high-grade SIL (HGSIL; according to the Bethesda system [Bethesda system, 1989]) with condylomatous lesions, were taken with the Cervex brush by turning the brush in the cervical canal seven times. The tip of the brush was broken from the holder and immersed in CytoRich™ preservation fluid. The cells were isolated as described recently [Geyer et al., 1993]. Briefly, cellular fragments and debris were removed by centrifuging the sample through a gradient density medium (2 min at 1,000 rpm in a Beckman benchtop centrifuge). The intact cells were harvested by a second centrifugation step (10 min at 2,000 rpm). The cell pellet was washed once with 10 mM Tris-HCL, pH 8.0, 1 mM EDTA (TE) and resuspended in TE before a small aliquot (usually 2 μl, equivalent to 0.5–1 × 10^5 cells) was used for PCR analysis.

HPV-PCR

To amplify HPV-specific sequences from formalin-fixed material, nested PCR was carried out [Adams et al., 1995]. Briefly, the primers MY09 and MY11 were used for the first amplification round to target a 450 bp product in the L1 open reading frame [Ting and Manos, 1990]. The sequences of the primers were as follows: MY09, 5′-CGTCCMARRGGAWACTGATC-3′ (M=A or C; R=A or G; W=A or T) and MY11, 5′-GCMCAGGG-WCATAAYAATGG-3′ (M=A or C; W=A or T; Y=C or T). PCR cycling conditions were: 30 sec denaturation at 96°C, 30 sec annealing at 50°C, and 1 min extension at 72°C. After 35 cycles an aliquot (5 μl) of the first PCR reaction was used as a template for the nested PCR amplification (total volume 50 μl). The following three primers were used simultaneously for the second round of amplification: MY09 (the same primer as used in the first amplification round); GP1, 5′-CTGTTGTTGAR-ACTACACGCAGTAC-3′ (R=A or G), and GP2, 5′-CT-GTGGTAGATACCACWCGCAGTAC-3′ (W=A or T). The PCR conditions were the same as for the first round of amplification.

To detect HPV-specific sequences in cells isolated from cervical smears, an aliquot (2 μl) of the cells was used directly as a DNA template in a PCR reaction containing the L1-specific consensus primers MY09 and MY11 [Manos et al., 1989].

The PCR product was electrophoresed on a 1% agarose gel and stained with ethidium bromide. Positive controls consisted of DNA extracted from tissues infected with HPV, as determined by ISH. Negative controls contained all PCR reagents without a DNA template.

HPV Typing

To distinguish between different HPV types aliquots of the amplification product were digested with five different restriction enzymes (BamH1, EcoR1, Hpa2, Hinf1, and Pst1). The fragments were separated on a 2% agarose gel and stained with ethidium bromide. The ability of a panel of restriction enzymes to digest or not to digest the PCR fragment was used to determine the exact HPV type (Table I). This procedure allows the discrimination between HPV-6b,11,16,18,31/33,45, 51, and 35/56. To confirm the results obtained by this restriction typing method, the PCR product was sequenced.

ISH

ISH was carried out according to a published protocol [Hassam et al., 1990] using three sets of commercially

TABLE I. Restriction Enzyme Panel Used To Determine the Exact HPV Type*

	EcoR1	BamH1	Hpa2	Hinf1	Pst1	EMBL accession no.
HPV-6b				+		X 00203
HPV-11		+		+	+	M 14119
HPV-16	+				+	K 02718
HPV-18		+	+		+	X 05015
HPV-18 provirus		+		+		M 73258
HPV-31/HPV-33				+	+	J 04353/M 12732
HPV-35/HPV-56					+	M 74117/X 74483
HPV-45		+			+	X 74479
HPV-51		+	+			M 62877
HPV-53	+			+		X 74482

*+, the restriction enzyme is able to cut the PCR product.

available biotinylated DNA probes specific for HPV types 6/11, 16/18, and 31/33/51, respectively (Enzo Diagnostics Inc., New York).

Detection and Typing of HPV by PCR-Enzyme-Linked Immunosorbent Assay (ELISA)

A schematic drawing of the method is shown in Figure 1. An aliquot of isolated cervical cells (2 μl) was added directly to the PCR reaction containing L1 consensus primers MY09/MY11, which were modified chemically at their 5′ ends; MY09 was labeled with digoxigenin and MY11 was biotinylated. To avoid the generation of nonspecific amplification products and primer-dimer formation, a hot start PCR protocol using wax beads (Perkin Elmer Cetus) was used. Furthermore, the annealing temperature of the modified primers was increased by 3°C. The PCR conditions were: 30 sec denaturation at 96°C, 1 min annealing at 53°C, and 40 sec extension at 72°C, 35 cycles.

In the case of HPV detection 10 μl of the PCR reaction was diluted with 40 μl of 0.5% blocking reagent (Boehringer Mannheim) in 1× phosphate-buffered saline (PBS). This mixture was added to a streptavidin-coated microtiter plate (Boehringer Mannheim) and incubated for 30 min at room temperature (RT). After washing the plate twice with 1 × PBS, a 1:3,000 diluted anti-digoxigenin antibody coupled to alkaline phosphatase (diluted in blocking buffer) was added to the plate. After incubation for 30 min at RT the wells were washed twice with 1× PBS and twice with 10 mM diethanolamine (pH 9.5) containing 0.5 mM $MgCl_2$. Fifty microliters of the substrate solution (1 mg/ml p-nitrophenyl phosphate [PNPP] in 10 mM diethanolamine [pH 9.5] containing 0.5 mM $MgCl_2$) was added to each well of the microtiter plate and incubated for several hours at RT. The reaction was stopped by the addition of 50 μl stop solution (100 mM EDTA) and the absorbance was read with a microplate reader (Bio-Rad Laboratories) at 405 nm. In the case of HPV typing on the microtiter plate an aliquot of the PCR reaction was digested with the appropriate restriction enzyme before it was diluted with the 0.5% blocking buffer in PBS and added to the microtiter plate for the detection of the digoxigenin residue.

RESULTS

Detection of HPV and Subsequent HPV Typing by Nested PCR-Restriction Enzyme Analysis and ISH

To compare the sensitivity and accuracy of the PCR-based detection and typing method with conventional ISH, we analyzed 34 routinely fixed paraffin-embedded cervical biopsy samples for the presence of HPV (Fig. 2). Using the nested PCR method HPV was detected in 11 of 34 cases (32%), whereas with ISH only 8 of 34 cases (23%) gave a positive staining. In 25 of the 34 (73%) cases investigated the ISH results are in accordance with results obtained by PCR. In five cases (15%) ISH showed a negative hybridization result whereas by PCR analysis the virus was detectable and the exact type was determined (Fig. 2 no. 6,12,14,17,32). In two cases (6%) the ISH gave a positive hybridization result, which could not be confirmed by PCR (Fig. 2 no. 15,23). In two other cases (6%; Fig. 2 no. 24,28) the presence of HPV was detected with both methods, but the HPV type was different (HPV-16/18 by ISH vs. HPV-45 by PCR and HPV-31/33/51 by ISH vs. HPV-18 provirus by PCR). HPV-45 and HPV-18 provirus was confirmed by direct sequence analysis of the PCR product. To detect HPV in the biopsy samples a nested PCR reaction was carried out, because after carrying out a single PCR reaction using the L1 primers MY09/MY11 none of the samples revealed a positive band on the ethidium bromide-stained agarose gel.

Typing of HPV Double Infections Using the Enzyme Digestion Method

In one case of the 34 biopsy samples a double infection was detected by ISH (Fig. 2 no. 4). The tissue was positive for HPV-6/11 (Fig. 3A), HPV-16/18 (Fig. 3B), and negative for HPV-31/33/51 (Fig. 3C). Analyzing the PCR product of this sample with the restriction typing method, the result shown in Figure 3D was obtained. The analysis of these restriction enzyme digests revealed that the enzyme Pst1 is able to digest the PCR product completely, whereas Hpa2 does not digest the fragment. Using Hinf1 or BamH1 most of the PCR product is digested, whereas with EcoR1 most of the product is not. This pattern is explained by a double

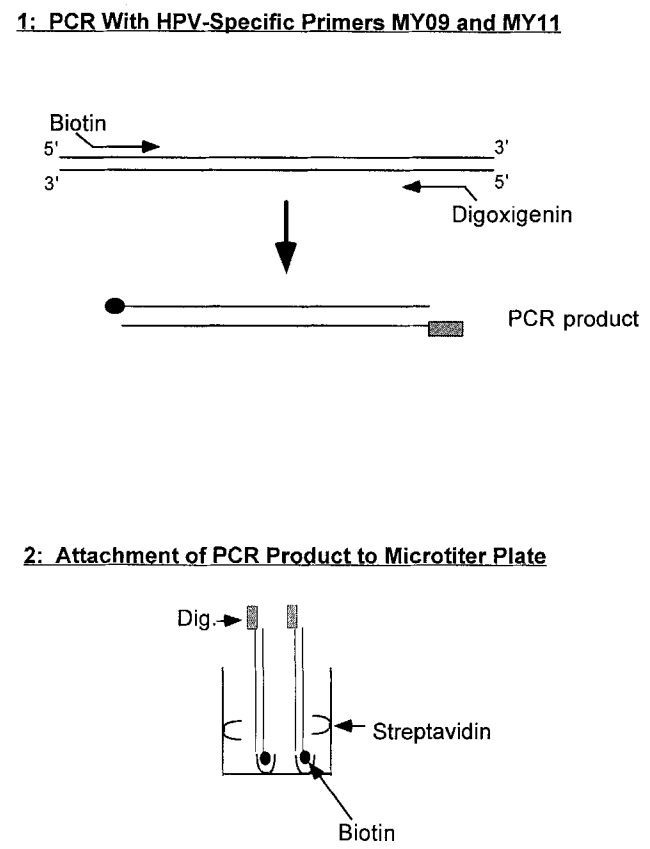


Fig. 1. Schematic drawing of the PCR-ELISA-based detection of HPV. The PCR is run with biotin and digoxigenin-modified primers. The amplification product is bound to a streptavidin-coated microtiter plate via the biotin molecule, and the digoxigenin moiety is detected with anti-digoxigenin antibodies coupled to alkaline phosphatase (AP).

infection with HPV-11,16. Furthermore, it is possible to predict that the amount of HPV-11 is higher than that of HPV-16, which is in accordance with the ISH results showing more positively stained cells using the probe for HPV-6/11 (Fig. 3A) compared to the probe for HPV-16/18 (Fig. 3B).

Detection and Typing of HPV in Cytological Specimens

To investigate whether the method described for HPV detection and typing can also be applied to cells isolated from cervical smears, we examined a group of 11 cytological normal and 11 HGSIL specimens for the presence of HPV (Table II). HPV was only detected in the group of patients with HGSIL (7 of 11; 63%) and not in the normal control group (0 of 11). Typing analysis revealed that four of seven specimens carried HPV-16, whereas only single cases of HPV-6b, HPV-35, and HPV-53 were found. HPV-35 and HPV-53 were confirmed by direct sequence analysis of the PCR product.

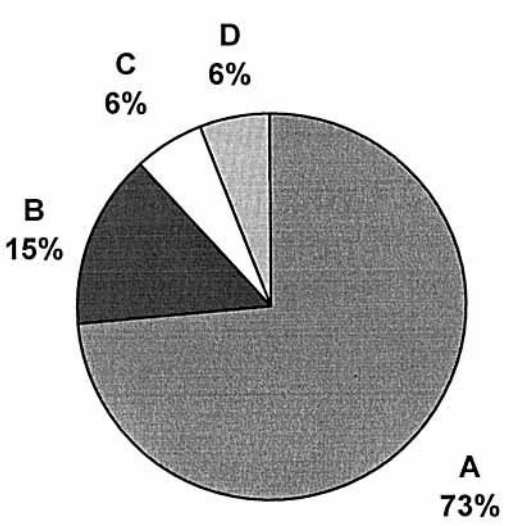
Detection and Typing of HPV on Microtiter Plates

To establish the detection of HPV by the PCR-ELISA-based system, we investigated the 22 cervical smear samples already examined by PCR followed by gel electrophoresis (Table II). The samples with a positive band on the agarose gel (Fig. 4A) also exhibited an OD_{405} value above the background level (Fig. 4B); cutoff $OD_{405} = 0.2$. Using this cutoff value the only questionable case is sample "995", which is diagnosed HPV negative by gel electrophoresis, but exhibits an OD_{405} value of 0.206. To demonstrate the feasibility of exact typing on the microtiter plate, we determined the exact HPV type of sample "992" by gel electrophoresis (Table II) and with the microtiter plate (Fig. 4C). Both methods revealed HPV-16. From these results it is clear that the microtiter plate assay is in good agreement with the results obtained by the conventional method using agarose gel electrophoresis.

DISCUSSION

The association between HPV and the development of cervical cancer suggests that targeted HPV screening represents an additional strategy for identifying women at high risk of developing cervical neoplasia [Morrison et al., 1991; zur Hausen, 1988]. The knowledge that the risk of developing a cervical neoplasia is restricted only to a subset of HPV types (zur Hausen, 1988; Lorincz et al., 1987; DeVilliers et al., 1987], the so-called high-risk HPVs, indicates that specific HPV typing may also be of prognostic value. Of these HPV-16 and 18 have been consistently the most prevalent oncogenic HPV types detected in cervical cancer tissue [Fuchs et al., 1988; zur Hausen, 1988; McCane et al., 1985; Boshart et al., 1984; Dürst et al., 1983]. Follow-up studies of patients with cervical intraepithelial neoplasia (CIN) II suggested that HPV-16-associated lesions are more likely to persist or to progress than those infected with HPV-6/11 [Syrjanen et al., 1985]. The observation that HPV-18 was detected 2.6 times more often within invasive cancer that occurred within 1 year of a negative smear [Lorincz et al., 1992] supports the view that HPV-18-infected cervical tissue progresses faster from normal to cervical carcinoma than squamous epithelium infected with HPV-16.

No	In-Situ Result	PCR Result
1	neg	neg
2	neg	neg
3	neg	neg
4	6/11 and 16/18	11 and 16
5	31/33/51	33
6	neg	18 pro.
7	31/33/51	33
8	neg	neg
9	neg	neg
10	neg	neg
11	neg	neg
12	neg	16
13	neg	neg
14	neg	16
15	6/11	neg
16	neg	neg
17	neg	56
18	neg	neg
19	neg	neg
20	neg	neg
21	neg	neg
22	neg	neg
23	16/18	neg
24	16/18	45
25	neg	neg
26	31/33/51	33
27	neg	neg
28	31/33/51	18 pro.
29	neg	neg
30	neg	neg
31	neg	neg
32	neg	18 pro.
33	neg	neg
34	neg	neg



A: ISH = PCR
B: ISH neg. / PCR pos.
C: ISH pos. / PCR neg.
D: ISH pos. / PCR pos. but different HPV type

Fig. 2. Comparison of HPV detection and typing by PCR/restriction enzyme analysis and ISH.

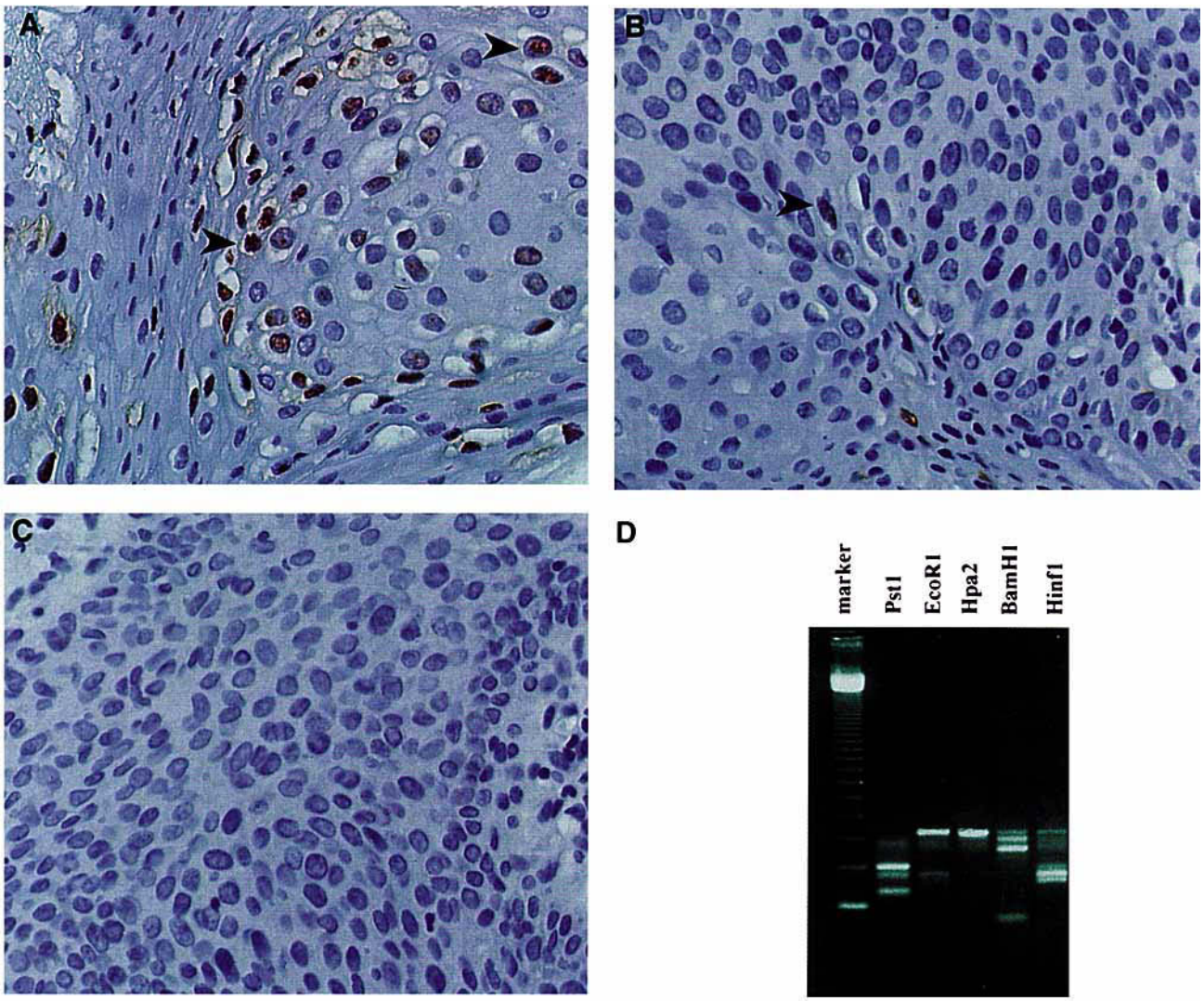


Fig. 3. ISH and PCR analysis of case no. 4. ISH on a section of cervix tissue with **A:** HPV probe 6/11; **B:** HPV probe 16/18; or **C:** HPV probe 31/33/51. ×300. **D:** Aliquots of the PCR amplification product were digested with different restriction enzymes and separated on a 2% agarose gel. The bands were visualized with ethidium bromide.

Several methods, such as Southern blot analysis, ISH, PCR, and in situ PCR, are described for the detection and typing of HPV [reviewed by Roman and Fife, 1989]. Each of these methods has its own advantages but also disadvantages compared to the others. For example, the most sensitive method to detect HPV is in situ PCR [Bernard et al., 1994a; Nuovo et al., 1991], but this method may not be applicable for screening a large number of specimens routinely. Our study and others [Delvenne et al., 1994; Popper et al., 1994] showed that the detection of HPV by nested PCR is more sensitive compared to conventional ISH using commercially available probes. In our panel of 34 formalin-fixed paraffin-embedded cervical samples we detected HPV in 11 of 34 cases (32%) by PCR with L1 consensus primers, whereas with ISH only 8 of 34 cases (23%) stained positive. The discrepancy of the detection rate between PCR and ISH is due in part to the fact that the L1 consensus primers detect a larger spectrum of HPVs than the conventional ISH. In most reports, a set of probes against HPV-6,11,16,18,31,33, and 51 are used for ISH, whereas the consensus primer system detects at least 27 different HPV types including most of the 20 types that have been isolated from genital tract lesions [Schiffman et al., 1991; Ting and Manos, 1990]. The other factor responsible for the discrepancy in the detection rate is the different sensitivity of both methods. A recent report by Bernard et al. [1994a] showed that at least 20–50 viral copies per cell are required to achieve a positive staining with ISH. With PCR 10–20 copies of the virus are enough to give a positive result [Shibata et al., 1988]. The increased spectrum of HPV types and the increased sensi-

TABLE II. HPV Detection and Typing in Cells Isolated From Cervical Smears

Case no.	HPV detection by PCR	HPV type	Cytological diagnosis
995	Negative		Normal
987	Negative		Normal
920	Negative		Normal
936	Negative		Normal
400	Negative		Normal
402	Negative		Normal
885	Negative		Normal
938	Negative		Normal
973	Negative		Normal
942	Negative		Normal
931	Negative		Normal
403	Negative		HGSIL
1008	Positive	16	HGSIL
414	Positive	16	HGSIL
982	Positive	35	HGSIL
992	Positive	16	HGSIL
985	Positive	53	HGSIL
999	Positive	6b	HGSIL
404	Negative		HGSIL
405	Positive	16	HGSIL
413	Negative		HGSIL
415	Negative		HGSIL

tivity favor the PCR method over ISH to detect HPV in fresh or paraffin-embedded tissue material.

To type specifically the detected HPV, several methods have been reported [Roman and Fife, 1989]. ISH often uses three sets of different probes to group the virus in high or low-risk type. Other approaches using a PCR strategy determine the exact HPV type either by blotting the PCR fragment generated with consensus primers and type-specific hybridization [Herrington et al., 1995; Lambropoulos et al., 1994] or by carrying out a type-specific PCR [Soler et al., 1991; Cuzick et al., 1994].

The restriction typing method described now, PCR amplification of HPV using consensus primer and subsequent digestion with a panel of different restriction enzymes, has several advantages compared with the other methods. By using consensus primers in the PCR reaction, a large spectrum of possible HPV types is covered without restricting the analysis to certain specific types, as is the case with type-specific primers. The determination of the specific HPV type depends on the ability of restriction enzymes to digest the generated PCR fragment. Because there is no need to depend on the generated banding pattern to determine the exact type [Bernard et al., 1994b; Chen et al., 1993, 1994; Pizzighella et al., 1993; Lungu et al., 1992; Fujinaga et al., 1991], the digested DNA can be separated on a normal 1–2% agarose gel, and not on a high-resolution polyacrylamide gel or on a 3% NuSieve gel. Using this strategy it is also possible to type specifically samples infected with two different HPV types (Fig. 3D), and furthermore it seems feasible to predict the viral load of one HPV type compared to the other.

Using the panel of five different restriction enzymes we were able to discriminate among 10 different HPV types. The distinction between HPV-31 and 33, which exhibit the same pattern with the described enzyme panel, is possible by using the restriction enzyme Hae3, which cuts the PCR product of HPV-31 but not of HPV-33. Kpn1 can be used to discriminate between HPV-35 and 56, so that with these additions up to 12 different HPV types are discriminated from each other. In instances where the digestion pattern does not fit into the described panel, the PCR product must be sequenced to determine the exact HPV type.

Methods are described allowing the detection of HPV with a PCR-ELISA system [Cox et al., 1995; Lungu et al., 1995]. The drawback of these methods is that for the determination of the exact HPV type a dot blot or restriction fragment length polymorphism (RFLP) analysis must be undertaken. The HPV typing system described in this report allows a transfer of the detection and the typing of HPV to a microtiter plate-based assay. To avoid nonspecific amplification products during the PCR reaction, specific precautions should be considered. It was observed that, despite the addition of modifying molecules to the primers, increasing the annealing temperature by 3°C and using a hot start reaction with wax beads circumvented the problem of nonspecific amplification. When this protocol was not used, nonspecific amplification products and primer dimer formation were observed leading to false-positive results with the ELISA detection system. In our series of samples we did not experience the problem of well-to-well cross-contamination on the microtiter plate. Analyzing the 22 cervical samples by PCR with subsequent gel electrophoresis (Fig. 4A) or by PCR-ELISA (Fig. 4B), the same results were obtained by both methods. The observation that not all HGSIL are HPV positive is in accordance with previous reports [Cornelissen et al., 1992; Lorincz et al., 1992; Fuchs et al., 1988]. The HPV prevalence of 63% in HGSIL may be underestimated, because Noffsinger et al. [1995] showed that the L1 primer system is less sensitive than primers in the E6 or E7 region of the virus. This difference may be related to L1 gene loss in cells containing integrated HPV-DNA [Stoler et al., 1992; Wagatsuma et al., 1990]. Typing sample "992" on the microtiter plate (Fig. 4C) revealed HPV-16 which agrees with the result obtained by PCR/restriction digest followed by agarose gel electrophoresis (Table II). This result demonstrates that the PCR-ELISA-based system allows the detection and typing of HPV infection in cytological specimens.

The advantage of the microtiter plate-based assay is that it is now possible to screen a relative large number of samples in a reasonable time. Furthermore, it will be feasible to establish a detection system capable of virus quantification. To quantitate the viral amount in a sample, the OD_{405} readout must be compared to a standard curve established with known amounts of HPV-DNA used as a PCR template. To speed up the whole procedure and to reduce manual handling, it may be possible in the future to run PCR with the modified primers in streptavidin-coated microtiter plates in which the PCR product can be detected later.

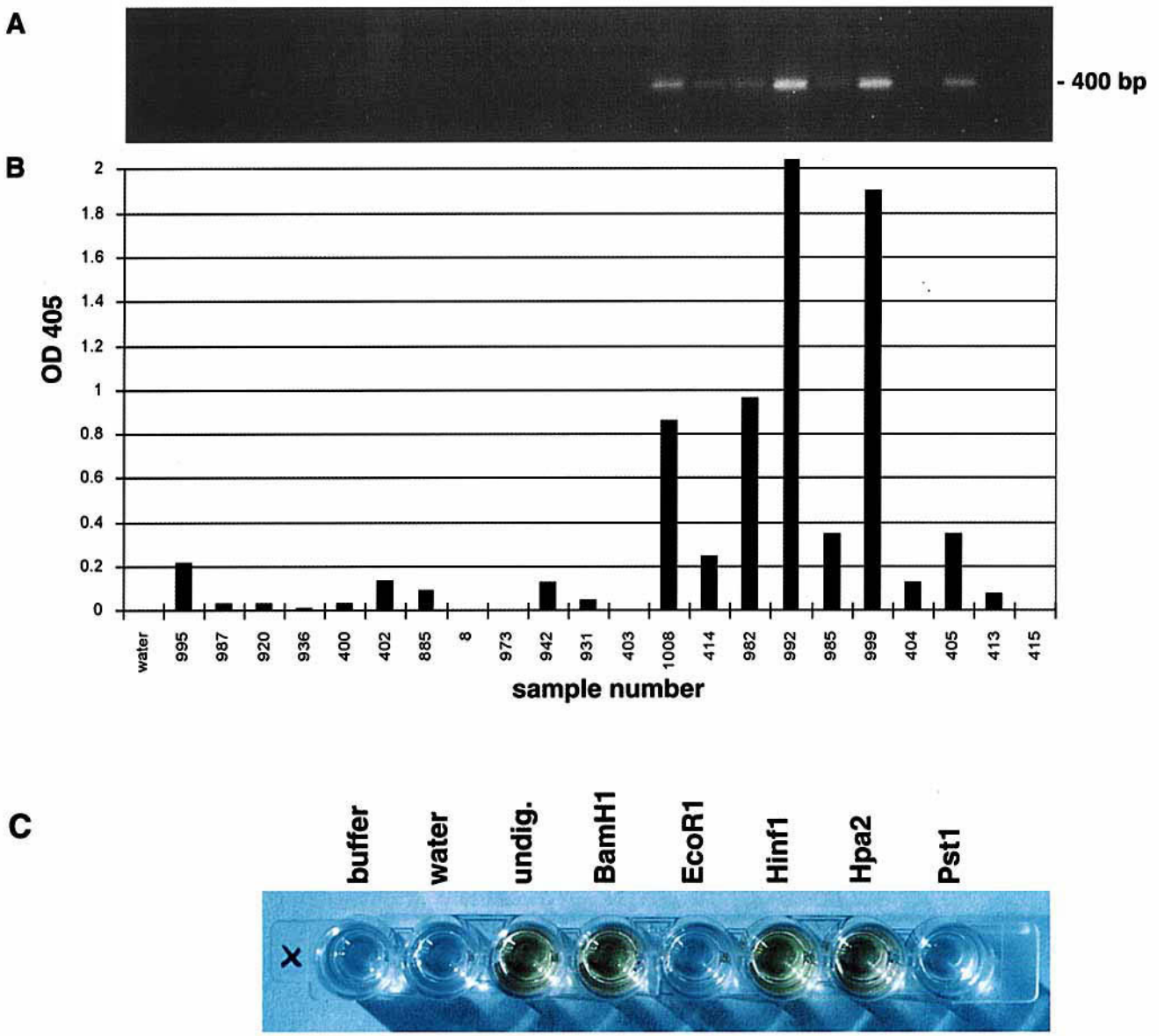


Fig. 4. Detection of HPV by PCR and gel electrophoresis or by the PCR-ELISA assay. **A:** Aliquots of the PCR amplification product are separated on a 2% agarose gel and visualized with ethidium bromide. The size of the specific amplification product is marked. **B:** An aliquot of the amplification product was bound to the streptavidin-coated microtiter plate and the digoxigenin molecule was detected with anti-digoxigenin antibodies. The OD_{405} of the color reaction was determined. The number of the samples is shown at the bottom. **C:** Sample "992" was predigested with the different restriction enzymes and added afterwards to the microtiter plate. The digoxigenin residue was detected. A yellow color reaction was seen if the restriction enzyme is not able to cut the PCR amplification product. undig. = undigested PCR amplification product.

This new technology and the progress made in cytology, isolation of cells from a conventional cervical smear, provide a routine screening method for HPV types in order to detect women with a higher risk of developing a cervical neoplasia. The system using a PCR-ELISA to detect viral infection is not restricted to HPV, but can easily be adapted to all other PCR-based detection systems [Zhang et al., 1994; McCabe et al., 1995; Zambardi et al., 1995]. Because of the lower amplification efficiency from formalin-fixed paraffin-embedded material, further investigations will be necessary to adjust the HPV PCR-ELISA system to fixed and embedded tissue material.

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